Comparison of bronchodilating and antiinflammatory activities of oral formoterol and its (*R,R*)-enantiomers[1]

XIE Qiang-Min[2], CHEN Ji-Qiang, SHEN Wen-Hui, YANG Qiu-Huo, BIAN Ru-Lian

Zhejiang Respiratory Drugs Research Laboratory of State Drugs Administration of China, Medical School of Zhejiang University, Hangzhou 310031, China

KEY WORDS formoterol; bronchodilator agents; inflammation; eosinophils; asthma; guinea pigs; mice

ABSTRACT

AIM: To compare the bronchodilating and antiinflammatory effects of oral racemic formoterol (rac-FMT) and (*R,R*)-formoterol (*R,R*-FMT). **METHODS:** The changes of lung resistance (R_L), dynamic lung compliance (C_{dyn}), and the accumulation of inflammatory cells in bronchoalveolar lavage fluids (BALF) induced by ovalbumin aerosol in sensitized guinea pigs and mice were investigated *in vivo*. **RESULTS:** Mean value increase of R_L and mean value reduction of C_{dyn} from 1 to 30 min after antigen challenge were up to 101 %±34 % and 42 %±7 %, respectively. rac-FMT 0.5, 1.0, and 2.0 mg/kg, and *R,R*-FMT 0.25, 0.5, and 1.0 mg/kg ig, induced dose-related inhibition of the bronchoconstrictive responses to aerosolised ovalbumin. ID_{50} (95% confidence limits, 95 % CL) value of rac-FMT on R_L maximal increase and C_{dyn} maximal reduction at 5 min were 0.64 (0.54-0.76) and 1.02 (0.88-1.18) mg/kg, respectively. For *R,R*-FMT they were 0.46 (0.40-0.53) and 0.52 (0.45-0.61) mg/kg, respectively. ID_{50} (95 % CL) value of rac-FMT on R_L mean increase and C_{dyn} mean reduction from 1 to 30 min were 0.96 (0.86-1.07) and 1.59 (1.32-1.92) mg/kg, respectively. For *R,R*-FMT they were 0.52 (0.45-0.59) and 0.43 (0.37-0.51) mg/kg, respectively. Ovalbumin-aerosol challenge induced an increase of inflammatory cells in BALF in sensitized mice. rac-FMT and RR-FMT caused a dose-dependent and almost complete inhibition at 2.0 mg/kg. ID_{50} (95 % CL) of rac-FMT on the number of total inflammatory cells and eosinophil in BALF were 1.48 (1.22-1.81) and 0.80 (0.62-1.04) mg/kg, respectively. ID_{50} (95 % CL) of *RR*-FMT were 0.80 (0.57-1.13) and 0.60 (0.43-0.83) mg/kg, respectively. **CONCLUSION:** *R,R*-FMT protected lung against increase of R_L and reduction of C_{dyn} induced by bronchial challenge of ovalbumin in the asthma model of guinea pigs, and inhibited airway inflammation in the sensitized mice. Efficacy of *R,R*-FMT was approximately 2-fold than that of rac-FMT.

INTRODUCTION

Racemic formoterol (rac-FMT) is a long acting β_2 adrenoceptor agonist which is used as a bronchodilator agent in asthma and chronic obstructive pulmonary disease[1,2]. The marketed form of formoterol is a racemic mixture composed of a 50:50 mixture of *R* and *S* isomers. Rac-FMT activity resides in the (*R,R*)-enantiomer whilst the (*S,S*) form is essentially inactive. Accordingly, pure (*R,R*)-formoterol (*R,R*-FMT) provides bronchodilation at lower doses than racemate, allowing for fewer β_2-adrenergic-mediated side effects. In addition, different metabolism may allow for the progressive accumulation of (*S,S*)-formoterol *S,S*-

[1] Project supported by the National Natural Science Foundation of China, No 39970857.
[2] Correspondence to Dr XIE Qiang-Min.
Phn 86-571-8721-7380. Fax 86-571-8721-7380.
E-mail xieqm@zju.edu.cn
Received 2002-03-04 Accepted 2002-08-05

FMT)[3]. The therapeutically active, long-acting β_2 agonist R,R-FMT is currently being developed according to the logic, and preliminary results suggest rapid improvements in forced expiratory volume in first second (FEV_1) with up to 24-h duration of action in the clinical test[4]. Pharmacological activities of rac-FMT and *R,R*-FMT have been compared; all have been *in vitro* involving airway tissue from horse[5], guinea-pig, and man[6] except anti-bronchoconstrictive activities of inhaled rac-FMT and *R,R*-FMT in the rhesus monkey *in vivo* recently[7]. We have compared the bronchodilating effect of rac-FMT and *R,R*-FMT on Chinese bronchus *in vitro* (unpublished data made by our Lab). In the present study, we compared the bronchodilating and antiinflammatory activities of oral rac-FMT and *R,R*-FMT in asthma model *in vivo*.

MATERIALS AND METHODS

Animals Hartley guinea pigs of either sex weighing (315±*s* 22 g) and NIH male mice weighing (22.5± *s* 1.5 g) were perchased from Laboratory Animal Center of Medical School of Zhejiang University (Grade II, Certificate No 220010014 conferred by Zhejiang Medical Laboratory Animal Administration Committee).

Drugs Rac-formoterol (purity >99.9 %) and *R,R*-formoterol (enantiomeric purity >99.6 %) were synthesized at Tianjing Drugs Research Institute; egg albumin (Reagent grade II and V, Sigma, USA); pentobarbital sodium (Union, Belgium); heparin sodium (Xuzhou Biochemical Pharmaceutical Factory).

Ovalbumin-induced changes of R_L and C_{dyn} in sensitized guinea pig

Sensitizing procedures Guinea pigs were sensitized by a single intraperitoneal injection (ip) of ovalbumin (grade II)10 mg mixed with 100 mg of aluminum hydroxide in saline 1.0 mL per animal. These animals were used 25 - 35 d later for aerosol challenge with ovalbumin.

Treatment procedures To determine dose-effect, in our preliminary experiments, guinea pigs were administered with rac-FMT and *R,R*-FMT of 0.125, 0.25, 0.5, 1.0 , 2.0, and 3.0 mg/kg by intragastric gavage (ig). To determine time-effect, we referenced human pharmacokinetic parameter of oral rac-FMT[8] and time-effect relation of animal pharmacodynamic parameter of intravenous injection (iv) rac-FMT[9]. Finally, the dose-ranges were definited with rac-FMT 0.5, 1.0, and 2.0 mg/kg or *R,R*-FMT 0.25, 0.5, and 1.0 mg/kg at 1.0 h before antigen challenge.

R_L and C_{dyn} measurement The sensitized guinea pigs were anesthetized with pentobarbital sodium (30 mg/kg) and placed in a whole body plethysmograph for the measurement of R_L and C_{dyn}[10]. The sensitized animals were treated with rac-FMT, *R,R*-FMT, and saline 1.0 h before a 1-min exposure to an aerosol of ovalbumin (grade II) 5.0 g/L in saline which was generated in a jet nebulizer (particle size 1-5 mm; BARI, MASTER, Germany). After antigen challenge R_L and C_{dyn} were monitored for 30 min and maximal changes from baseline for each parameter were recorded by a Medlab (V 5.0, Medease Scientific Technic Co Ltd, Nanjing). The effect of rac-FMT or *R,R*-FMT was determined by comparing the ovalbumin-induced changes in R_L and C_{dyn} after drug treatment with the mean of antigen responses alone in the same guinea pig on previous and successive control periods.

Ovalbumin-induced airway inflammation in sensitized mice

Sensitizing procedures NIH mice were sensitized by subcutaneous injection (sc) at d 0 with antigen ovalbumin (grade V) 1 mg mixed with aluminium hydroxide adjuvant 50 mg in saline 0.2 mL, at footpad, neck, back, and groin. Control animals received saline at the same points[11].

Treatment procedures One time every day for 7 d and 1 h before antigen challenge, treatment mice were administered with rac-FMT or *R,R*-FMT 0.5, 1.0, and 2.0 mg/kg at 1.0 h before antigen challenge. Challenged control mice were injected ig with saline.

Antigen challenge After treatment, mice were placed in a 4-L bell cover and challenged by exposure to an aerosol of ovalbumin 10 g/L in saline which was generated in a jet nebulizer for 20 min, and one time every day for 7 d. Control mice were similarly exposed to an aerosol of saline.

Cell recovery and counts From the airway lumen of mice at 24 h after the last time antigen challenge, mice were anesthetized with urethane 2 g/kg, ip, and the trachea was cannulated. Bronchoalveolar lavage was performed by flushing the airways with saline 1.0 mL per mouse containing 1 % bovine serum albumin and 1 kU/L heparin sodium through the tracheal cannula for three flushing. Counts of total number of inflammatory cells recovered in bronchoalveolar lavage fluids (BALF) were made using a Neubauer chamber. BALF was centrifuged (Eppendorf Centrifuge 5804R, Germany) at 500×*g* for 10 min at 4 °C, the cells deposit was resuspended in 0.2 mL saline containing

10 % bovine serum albumin, and smeared on a slide, differential cell analysis was made under light microscopy after Wright-Gieamsa staining.

Statistical analysis Data are expressed as mean±SD. A statistical analysis was performed using a one-way analysis of variance (Student-Newman-Keuls test). ID_{50} (95% confidence limits) was calculated and compared by weighted probit analysis of Bliss method.

RESULTS

Antigen-induced changes of R_L and C_{dyn} in sensitized guinea pigs Comparision with baseline value before aerosol challenge of antigen, bronchial challenge of ovalbumin-sensitized guinea pigs induced 140 % increase of R_L and 49 % reduction of C_{dyn} with maximal response at 5 min. Mean value increase of R_L and mean value reduction of C_{dyn} from 1 to 30 min after antigen challenge were up to 101 %±34 % and 42 %±7 %, respectively in saline group. rac-FMT 0.5, 1.0, and 2.0 mg/kg, administered by ig, induced dose-related inhibition of the bronchoconstrictive responses to aerosolised ovalbumin. Maximal increase of R_L and reduction response of C_{dyn} at 5 min were 86.0 %, 42.1 %, and 16.9 %, and 36.2 %, 28.7 %, and 9.6 %; ID_{50} (95 % CL) were 0.64 (0.54–0.76) and 1.02 (0.88–1.18) mg/kg, respectively. Mean increase of R_L and reduction response of C_{dyn} from 1 to 30 min were 62 %±16 %, 39 %±12 %, and 16 %±10 % and 32 %± 5 % , 19 %±5 %, and 14 %±2 %; ID_{50} (95 % CL) were 0.96 (0.86–1.07) and 1.59 (1.32–1.92) mg/kg, respectively (Fig 1). *R,R*-FMT, 0.25, 0.5 and 1.0 mg/kg administered by i g produced dose-related inhibition of the bronchoconstrictive responses to aerosolised ovalbumin. Maximal increase of R_L and reduction response of C_{dyn} at 5 min were 103.6 %, 68.6 %, and 24.7 % and 39.6 %, 21.9 %, and 13.6 %, ID_{50} (95 % CL) were 0.46 (0.40–0.53) and 0.52 (0.45–0.61) mg/kg, respectively. Mean increase of R_L and reduction response of C_{dyn} from 1 to 30 min were 79 %±24 %, 55 %±20 %, and 22 %±4 % and 29 %±9 % , 19 %± 4 %, and 9 %±3 %; ID_{50} (95 % CL) were 0.52 (0.45–0.59) and 0.43 (0.37–0.51) mg/kg, respectively (Fig 2).

Antigen-induced changes of airway inflammatory cells in BALF In sensitized mice, after 7× ovalbumin-aerosol challenge, antigen induced a significant increase of inflammatory cells in BALF. The number of inflammatory cells in BALF in antigen challenged

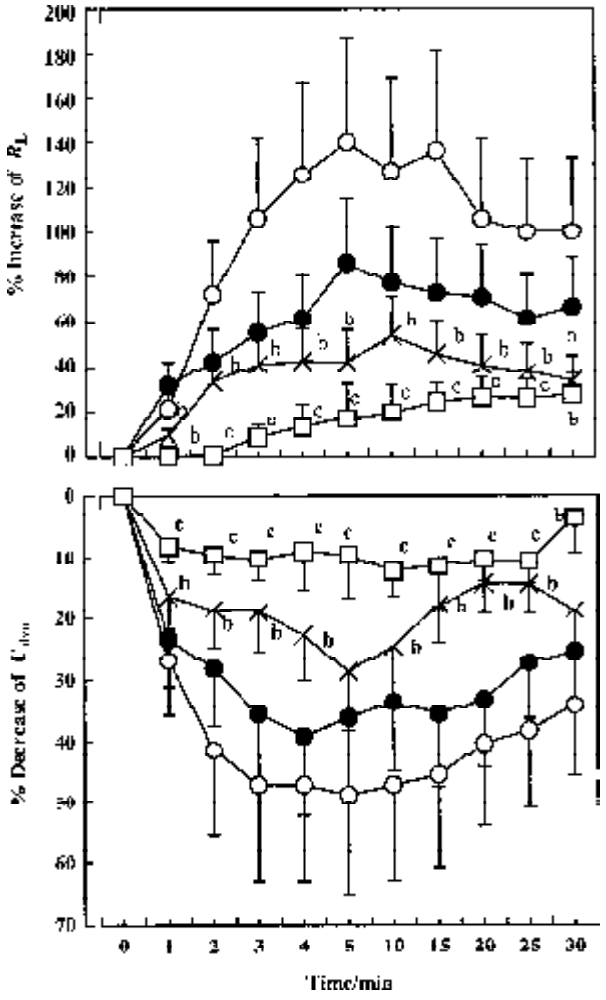

Fig 1. Effect of formoterol on lung resistance and dynamic lung compliance challenged with ovalbumin aerosol in sensitized guinea pigs. Saline (○), Rac-formoterol 0.5 mg/kg (●), Rac-formoterol 1.0 mg/kg (×), Rac-formoterol 2.0 mg/kg (□), ig 1 h before exposure to ovalbumin aerosol. *n*=8 guinea pigs. Mean±SD. $^bP<0.05$, $^cP<0.01$ *vs* saline.

group was significantly higher than that in control group ($P<0.01$). Rac-FMT and *R,R*-FMT caused a dose-dependent and almost complete inhibition at 2.0 mg/kg (Tab 1). ID_{50} (95 % CL) of rac-FMT on the number of total inflammatory cells, eosinophil, and lymphocyte and macrophage in BALF were 1.48 (1.22–1.81) , 0.80 (0.62–1.04), and 1.73 (1.45–2.05) mg/kg, respectively. ID_{50} (95 % CL) of *R, R*-FMT were 0.80 (0.57–1.13), 0.60 (0.43–0.83), and 0.79 (0.52–1.21) mg/kg, respectively (Tab 1).

DISCUSSION

Rac-FMT contains two chiral carbons and, therefore, the synthetic molecular product contains two enantiomers (*RR* and *SS*) and two diastereomers (*RS*

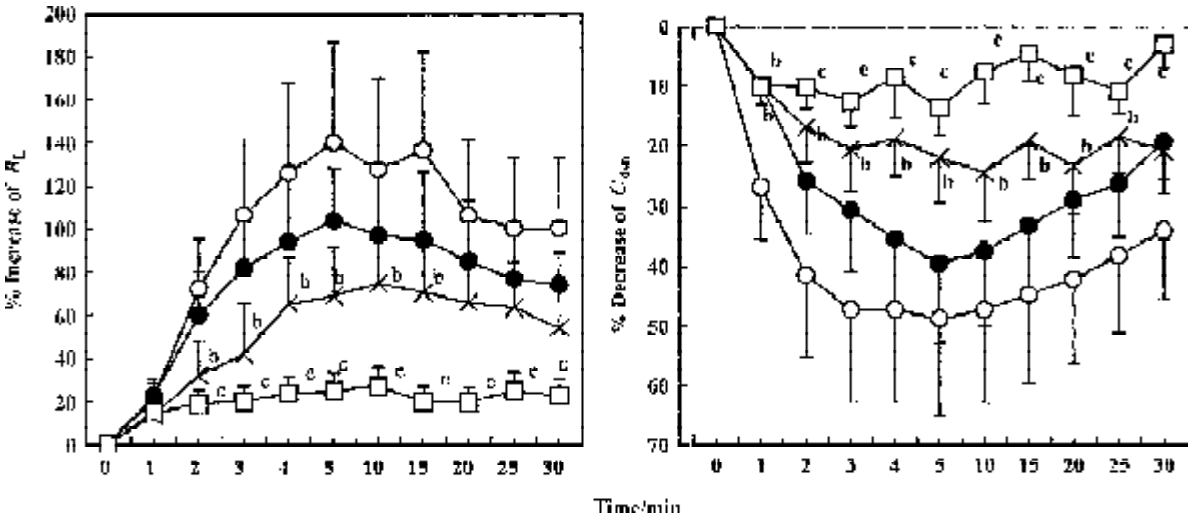

Fig 2. Effect of *R, R*-formoterol on lung resistance and dynamic lung compliance challenged with ovalbumin aerosol in sensitized guinea pigs. Saline (○), *R,R*-formoterol 0.25 mg/kg (●), *R, R*-formoterol 0.5 mg/kg (×), *R,R*-formoterol 1.0 mg/kg (□), ig 1 h before exposure to ovalbumin aerosol. *n*=8 guinea pigs. Mean±SD. $^bP<0.05$, $^cP<0.01$ *vs* saline.

Tab 1. Inhibition of antigen-induced lung inflammatory cells in bronchoalveolar lavage fluid by rac-formoterol and *R,R*-formoterol. Mean±SD. $^bP<0.05$, $^cP<0.01$ *vs* saline. $^eP<0.05$ *vs* rac-FMT 1.0 mg/kg.

Groups	Dose	*n*	$10^{-8}\times$ Total cells/L^{-1}	$10^{-8}\times$ Eosinophil/L^{-1}	$10^{-8}\times$ Neutrophil/L^{-1}	$10^{-8}\times$ Lymphocyte and macrophage/L^{-1}
Control		8	1.0±0.3^c	0.1±0.3^c	0.7±0.4^c	
Saline	10 mL/kg×7 d	10	4.4±2.2	1.6±0.6	0.8±0.7	2.0±0.9
rac-FMT	0.5 mg/kg×7 d	10	3.6±2.2	0.9±0.7^b	0.9±0.8	1.8±1.2
rac-FMT	1.0 mg/kg×7 d	10	3.0±1.6	0.8±0.6^b	0.6±0.9	1.6±0.5
rac-FMT	2.0 mg/kg×7 d	10	1.7±0.7^c	0.4±0.4^b	0.5±0.6^b	0.8±0.4^c
R,R-FMT	0.5 mg/kg×7 d	11	2.6±0.8^b	0.8±0.5^b	0.7±0.4	1.1±0.5^c
R,R-FMT	1.0 mg/kg×7 d	11	2.1±0.8^b	0.6±0.4^b	0.5±0.5^b	1.0±0.4^{ce}
R,R-FMT	2.0 mg/kg×7 d	10	1.5±0.5^c	0.3±0.2^c	0.5±0.3^c	0.7±0.5^c

and *SR*)[4]. The marketed product contains only the (*RR*)- and (*SS*)-non-superimposable enantiomers. Since rac-FMT is a 50:50 mixture of the two enantiomers, the distomer (*S,S*-FMT) is inactive at the same dose of the eutomer which showed activity and the eudismic ratio is close to 1000[6], the result is exactly to be expected. In our results, rac-FMT and *R,R*-FMT present dose-related inhibition of the bronchoconstrictive responses and airway inflammation to aerosolised ovalbumin in the asthma models of guinea pig and mouse. ID_{50} value of rac-FMT is approximately two-fold than that of *R,R*-FMT, which shows the bronchodilating and antiinflammatory effects of the eutomer, *R,R*-FMT, are qualitatively similar to those of racemic rac-FMT, that at a half dose of the racemate the eutomer has a similar effect. Thus, the bronchodilating and antiinflammatory effects of the racemate can be attributed entirely to the *R,R*-enantiomers. Waldeck found that the (*R,R*)-enantiomer was by far the most potent. However, the relative potencies obtained for the (*R,S*)-, (*S,R*)-, and (*S,S*)-isomers were critically dependent on the degree of enantiomeric purity[12]. The results suggest the relative potency as that of receptor binding affinities. Handley and his colleagues described the binding affini-

ties (k_d) of *R,R*-FMT and *S,S*-FMT for the β_2 receptor were 2.9 nmol and 3100 nmol, respectively, whereas the k_d for rac-FMT was 5.2 nmol. Therefore, *R,R*-FMT is 1000-fold than that of *S,S*-FMT and 2-fold than that of receptor-binding activity of rac-FMT[4]. However, the presence of the distomer in the racemate does not influence the bronchodilating and antiinflammatory activity of the eutomer. Similar findings were reported from *in vivo* anti-bronchoconstrictive activity in monkey[7] and, from *in vitro* studies with animal tissues[5,6] and, more recently, from an analysis of the relaxant activities of the enantiomers of rac-FMT against resting tone and high tone induced by histamine and carbamylcholine in isolated Eurasian[6] and Chinese bronchi (unpublished data made by our Lab). Collectively, the results indicate that *R,R*-FMT would offer no benefit over the racemic mixture as far as the bronchodilating and antiinflammatory activity is concerned except that the dose could be halved. However it should be emphasized, the possibility of disadvantages or adverse effects of the distomer cannot be ruled out here.

In summary *R,R*-FMT protects lung against increase of R_L and reduction of C_{dyn} induced by bronchial challenge of ovalbumin in the asthma model of guinea pig, and inhibits airway inflammation in the sensitized mouse. Efficacy of *R,R*-FMT is approximately two-fold than that of rac-FMT. The bronchodilating and antiinflammatory activity of rac-FMT resides in the *R,R*-enantiomers and *S,S*-enantiomers does not interfere with the activity when present in the racemic form.

REFERENCES

1 Bartow RA, Brogden RN. Rac-FMT. An update of its pharmacological properties and therapeutic efficacy in the management of asthma. Drugs 1998; 55: 303-22.

2 Cazzola M, Donner CF. Long-acting β_2 agonists in the management of stable chronic obstructive pulmonary disease. Drugs 2000; 60: 307-20.

3 Zhang M, Fawcett JP, Kennedy JM, Shaw JP. Stereoselective glucuronidation of rac-FMT by human liver microsomes. Br J Clin Pharmacol 2000; 49: 152-7.

4 Handley DA, Anderson AJ, Koester J, Snider ME. New millennium bronchodilators for asthma: single-isomer beta agonists. Curr Opin Pulm Med 2000; 6: 43-9.

5 Zhang XY, Zhu FX, Oiszewski MA, Robinson E. Effects of enantiomers of β_2-agonists on ACh release and smooth muscle contraction in the trachea. Am J Physiol 1998; 274: L32-8.

6 Schmidt D, Kaellstroem BL, Waldeck B, Branschied D, Magnusson H, Rabe K. The effect of the enantiomers of rac-FMT on inherent and induced tone in guinea-pig trachea and human bronchus. Naunyn-Schmiedeberg's Arch Pharmacol 2000; 361: 405-9.

7 Fozard JR, Buescher H. Comparison of the anti-bronchoconstrictor activities of inhaled rac-FMT, its (*R,R*)- and (*S,S*)-enantiomers and salmeterol in the rhesus monkey. Pulm Pharmacol Ther 2001; 14: 289-95.

8 van den Berg BTJ, Braat MCP, van Boxtel CJ. Pharmacokinetics and effects of formoterol fumarate in healthy human subjects after oral dosing. Eur J Clin Pharmacol 1998; 54: 463-8.

9 Mohammed SP, Taylor CV, Weyman-Jones CB, Mather ME, Vendy K, Dougall IG, *et al.* Duration of action of inhaled vs intravenous β_2-adrenoceptor agonists in an anaesthetized guinea-pig model. Pulm Pharmacol Ther 2000; 13: 287-92.

10 Xie QM, Zeng LH, Zheng YX, Lu YB, Yang QH. Brochodilating effects of bambuterol on bronchoconstriction in guinea pigs. Acta Pharmacol Sin 1999; 20: 651-4.

11 Xie QM, Chen JQ, Shen WH, Bian RL. Correlative changes of interferon-γ and interleukin-4 between cortical layer and pulmonary airway of the sensitized rats. Acta Pharmacol Sin 2002; 23: 248-52.

12 Waldeck B. Biological significance of the enantiomeric purity of drugs. Chirality 1993; 5: 350-5.